Environmental and Experimental Botany 150 (2018) 1–8

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Physiological and fitness response of flowers to temperature and water augmentation in a high Andean geophyte

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ARTICLE INFO

Keywords:
Conductance
Water stress
Actual flower longevity
Gas-exchange
Open-top chamber
Alpine

ABSTRACT

Flowers are likely to be under various selective pressures not limited to reproduction and resource trade-offs. Here, we investigate the physiological responses (conductance, transpiration and respiration) of flowers under augmented temperature and supplemental watering, link these responses to actual flower longevity, and search for evidence of adaptive plasticity. Flower maintenance costs and plant fitness were investigated using the Andean geophyte, *Rhodolirium montanum*, in a manipulative field experiment. Maintenance determined by gas exchange was measured on fully expanded tepals; along with flower duration and its components, opening and closing. Fitness measured by fruit and seed production and seed set (mature/total) was examined. We artificially increased temperature and supplementally watered plants. Our study showed that flower longevity decreased under drier conditions, but the effect on flower opening and closing was even stronger, both of which advanced under higher temperatures without supplemental watering. Gas exchange also decreased, limiting water loss, under drier, warmer conditions. Those plants that reduced flower longevity under warming had higher total seed production and seed set. Plants that reduced conductance under warmed conditions also had higher seed set. These results suggest that plants are plastically optimizing fitness by reducing flower maintenance costs. Possible flower longevity and fitness connotations of our results under climate change are discussed.

1. Introduction

Flower traits are considered to be shaped by natural selection to maximize ovule fertilization and pollen dispersal while minimizing maintenance costs (Ashman and Schoen, 1996, 1994). The cost to plants in maintaining showy, long-lived flowers may be significant. In an early study, Nobel (1977) showed that the final dry weight of the inflorescence of *Agave deserti* was equal to the photosynthetic productivity for the entire year of the mature plant. The respiratory costs of maintaining flowers open for several days may ultimately reduce resources for fruit production; in the annual, *Clarkia tembloriensis*, reduction in resource production with longer flower maintenance, resulted in lower seed production (Ashman and Schoen, 1997). These considerations suggest traits like flower longevity, the amount of time a flower remains open before and after pollination, will be fine-tuned over time, with selection favoring long-lived flowers so as to increase the probability of pollination, but with resource availability constraining direct positive selection. Here, we focus on the resource-cost hypothesis in alpine *Rhodolirium montanum*. *Rhodolirium montanum* is a large-flowered, showy geophyte with long-lived flowers (Arroyo et al., 2017; Pacheco et al., 2016). Studies in alpine environments suggest showy, long-lived flowers are favored to increase the probability of pollination under low visitation rates (Arroyo et al., 2017, 2013; Bingham and Orthner, 1998; Steinacher and Wagner, 2010; Torres-Díaz et al., 2011; Utelli and Roy, 2000). Thus, alpine species are interesting material for testing the resource-cost hypothesis given the generally adaptive value of long-lived flowers in the alpine environment and for studying the physiological processes that underpin the length of the flower life-span.

Water availability and temperature are important limitations for plant growth and reproduction. Flowers, especially if they are long-lived, represent a major draw on a plant's water reserves (Erickson and Markhart, 2002; Galen, 2005). Galen et al. (1999) suggested that for the alpine plant *Polemonium viscosum*, the negative cost associated with water loss from corollas influenced variation in corolla size by selecting for smaller corollas conversely to pollinator-mediated selection for larger corollas. There are two major routes of water loss for flowers: transpiration through stomata and evaporative loss from tissue surfaces. Stomata in flowers are not always functional (Hew et al., 1980); often, the guard cells do not close stoma as leaf guard cells might during high

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https://doi.org/10.1016/j.envexpbot.2018.02.015
Received 4 January 2018; Received in revised form 26 February 2018; Accepted 26 February 2018
Available online 27 February 2018
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vapor pressure deficits or drought stress. Thus, flowers are like "faucets" that plants are unable to turn off without amputating an organ. For example in avocado inflorescences, tepals transpire more than leaves under field conditions (Blanke and Lovatt, 1993). The open stomata may be adaptive under high temperatures, however, maintaining ovules and fertilized seeds at an optimum temperature through evaporative cooling (Erickson and Markhart, 2002; Galen, 2005).Therefore, water shortage sustained over time could prevent the normal development of flowers, affecting both fruit and seed production (Fang et al., 2010; Galen, 2005)

Flowers are especially sensitive to ambient temperature and temperature stress (Hedhly et al., 2009; Porter, 2005). Temperature stress has been shown to ultimately result in decreased yields in crop species (Porter, 2005). In two high-alpine species, *R. montanum* and *Oxalis compacta*, potential flower longevity measured in pollinator-excluded flowers decreased in response to experimentally raised temperatures (Arroyo et al., 2013; Pacheco et al., 2016). Water and temperature are known to interact to affect plant reproduction. For example, in corn, the negative effects of temperature on reproduction were increased at both extremes of water availability: deficit and excess (Hatfield and Prueger, 2015). The combined effect of temperature and water availability on alpine flowers is relatively unexplored, but could be especially pertinent to understanding ecologically-relevant traits like flower longevity that also have the potential to directly affect fitness, as well as on how climate change will affect reproductive processes. To date however, most work on flower longevity in the alpine has focussed on the effect of temperature (*e.g.* Arroyo et al., 2017, 2013; CaraDonna et al., 2014; Pacheco et al., 2016; Price and Waser, 1998; Telwala et al., 2013)

Here, we experimentally manipulate temperature and water availability, both key abiotic factors affecting flower maintenance, in the field in *R. montanum*. We hand pollinated all flowers within our study, thereby focusing our investigation on a plant's response and maintenance of flowers after pollination. Experiments have shown that flowers of *R. montanum* stay open and fresh for several days after pollination (Arroyo et al., 2017), making it an ideal species for testing the effect of water and temperature on flower longevity, and the effect of environmentally-determined variation in flower longevity on seed set. We measured actual flower longevity (flower longevity in pollinated flowers) under the water and temperature treatments along with several physiological traits related to flower maintenance: conductance, transpiration and respiration. We collected fruits from these experimentally manipulated flowers to estimate the effects of our treatments on plant fitness. We expect that if flowers are responding plastically to environmental conditions, pollinated flowers will minimize maintenance costs by having:

1. lower conductance, transpiration and respiration rates while simultaneously having shorter flower longevities under ambient compared to supplementally watered conditions
2. higher conductance, transpiration, and respiration rates while simultaneously having longer flower longevities under ambient compared to experimentally warmed conditions

In addition, if plants respond adaptively to water availability and temperature, we predict that they will maximize fitness by minimizing maintenance.

2. Materials and methods

2.1. Study species

Rhodolirium montanum Phil [syn: *R. rhodolirion* (Baker) Traub.] (Amaryllidaceae) is a large-flowered geophyte found between 2100–3300 m.a.s.l. in the central Chilean Andes. The species is strongly outcrossing and mainly pollinated by the native bee *Megachile sauleyi* (Guerin-Meneville) (Ladd and Arroyo, 2009). *Rhodolirium montanum* was selected for our study because it has relatively large flowers (51 +/− 7 mm, D. Pacheco unpublished data in the same population in 2015). At the time of anthesis all its leaves have dried up; consequently, all water loss during the summer months will be through flowers and their supporting peduncles. Each flowering stem (peduncle) bears a single flower (very rarely 2 pedicels per flowering stem). Flowers, on average, produce ~300,000 pollen grains and 45 ovules (Ladd and Arroyo, 2009). Previous work has shown flower duration to be variable with virgin flowers open on average 5.7-8.1 days (Pacheco et al., 2016). In addition, as the flower is nectar-less, maintenance cost of the flowers would not include this pollinator resource (Ladd and Arroyo, 2009). Finally, while flower senescence in *R. montanum* shows some response to pollination (Arroyo et al., 2017), early pollinated flowers continue to be turgid and remain open several days following ample hand-pollination. Thus, the effect of water and temperature can be assessed simultaneously on flower longevity and fitness.

2.2. Study site

During the austral summer (January–March) 2016, we monitored duration of flowering of *R. montanum* in a population located in the area of the Valle Nevado ski resort at 2267 m.a.s.l. (S 33°22′15.8″W 70°17′17.5″) in the central Chilean Andes. The first flower to open in the population was on 12 January 2016 and our last fruit collection from our experimental plants was on 28 March 2016.

2.3. Experimental design

Flower buds were chosen haphazardly across the more densely populated area of the site. Once four buds were chosen, they were randomly assigned to one of two treatments each with two levels: Water (supplementally watered, hereafter +water or no water addtion, hereafter–water) and Temperature (augmented by placing an Open-Top chamber over plants, hereafter +OTC or left open to ambient conditions, hereafter –OTC).Chambers were constructed from six polyethylene panels, each containing 15 holes approximately 1.5 cm in diameter and joined to each other at an angle so that the result was an open hexagon approximately 50 cm in height and 120 cm in diameter. Similar passive chambers have been used in other studies to experimentally warm plants (Henry and Molau, 1997, see for example Marion et al., 1997), including in the Chilean Andes (Sanfuentes et al., 2012; Arroyo et al., 2013). The supplementally watered treatment (+water) was accomplished by using a back-pack type sprayer (SOLO Sprayer 425) that sprayed a light mist out of the nozzle. We misted a 120 cm area around treatment plants daily prior to maximum radiation. This resulted in approximately 50 mL of supplemental water delivered everyday from the initiation of a flower bud until past flower senescence, starting 11 January 2016 and ending 16 February 2016. For the −OTC treatment, plants were placed in wire cages to reduce the likelihood of large mammal grazing.

Once a bud had been assigned to a treatment, buds that subsequently came up within a 120 cm radius of the first bud were also placed under the same treatment; thereby increasing the number of buds and subsequent flowers being observed for each treatment. In severe environments, plants mitigate the risk of mortality with clonal growth, where several ramets form a clump. We recognized this growth pattern by marking buds that were very close as the same genet with several ramets. Individuals that grew further from the colony were considered as independent genets.

During the flowering season, we monitored 377 genets and 486 flowers (107 −water −OTC, 109 +water −OTC, 149 −water +OTC, and 119 +water +OTC).

2.4. Ambient environment

Temperature and relative humidity were recorded every 15 min,

using 12 data loggers (HOBO U23 Pro v2; Onset Computer Corp., Cape Cod, MA, USA). Three loggers were randomly assigned to each treatment. Loggers were placed 15 cm above the soil surface and shaded by an inverted white cardboard cup. For each day, a mean was calculated for the minimum, maximum and average temperature and relative humidity for each data logger. These daily means were used for further analysis. The minimum value means for relative humidity were $\log_{10}$ transformed in order to meet normality assumptions prior to analysis. Two-way, full factorial analysis of variance (ANOVA) was conducted for each variable (six total) separately. A two-way interaction between Watering and Temperature was followed up with a Tukey's Honest Significant Difference test. All analyses here and below were performed using JMP Pro (v 12.0.1).

Soil moisture (% volumetric water content) was measured daily, using a soil probe (FIELDSCOUT TDR300 soil moisture meter Spectrum Technologies Inc.). One focal plant group per treatment was randomly selected without replacement for the duration of the experiment. The probe measured soil moisture within one meter of the focal plant group. Volumetric water content was $\log_{10}$ transformed in order to meet normality assumptions prior to analysis. An analysis of covariance (ANCOVA) was used to test Water and Temperature effects, their interaction and the covariate, day of year.

2.5. Flower duration

The day of year in which the flower opened (any portion of a sexual organ exposed, mainly opening crepuscularly) and closed (tepals lost turgor) were recorded. Flowers were monitored daily with rare exceptions due to unamenable weather conditions. For those exceptional cases, flower opening was estimated based on anther dehiscence status (first day of opening, anthers did not dehisce, second day of opening anthers had started to dehisce). All flowers being monitored were pollinated upon stigma lobe separation (usually the first day of opening, 19 instances in which stigmas were pollinated on the second day). Pollen was collected from five genetically distinct flowers outside of the treatment area but within the same population, pooled and used to pollinate several of the flowers until making stigma surfaces uniformly yellow from pollen. This procedure was repeated as neccessary until all newly receptive stigmas within our experimental population were pollinated. Flower duration was calculated as the day of flower closing minus the day of flower opening (based on day of year). Flower duration, flower opening and flower closing were analyzed using separate mixed model analyses of variance (mANOVA) in which Chamber and Water were fixed and permitted to interact. Random effects included experimental treatment group, genet nested within group, and individual flower stalk nested within group and genet.

2.6. Gas exchange measurements

One tepal per open flower was used to measure gas exchange. We used a portable infrared gas exchange analyzer (IRGA, LiCor 6400; LiCor, Lincoln, Nebraska, USA) equipped with a LiCor 6400-40 fluorometer light source to measure respiration rate ($\mu mol_{CO2}\, m^{-2}_{leafarea}\, s^{-1}$), transpiration rate ($mol_{H2O}\, m^{-2}_{leafarea}\, s^{-1}$) and conductance ($\mu mol_{H2O}\, m^{-2}_{leafarea}\, s^{-1}$). The tepals were too narrow to fill the IRGA's two cm^2 leaf chamber, and so an area correction was calculated based on tepal area inside the chamber. The area inside the chamber was marked by placing stamp ink on the chamber gasket, which marked the tepal. A clear glass slide that had been marked with the two cm^2 circular area was placed over the stamped area. A ruler was placed next to the slide and a digital image was taken. The image of each tepal was analyzed, using the program ImageJ (National Institutes of Health, Bethesda, Maryland, USA; available at http://rsbweb.nih.gov/ij) in order to determine the area that was exposed within the chamber. We then used the recompute utility of the LiCor 6400 to adjust gas exchange rates to reflect the actual tepal area measured within the chamber.

Gas exchange was recorded the first day of flower opening for one tepal per flower for a subset of the plants within each treatment. The following settings were used: photosynthetically active radiation (PAR_{in}) = 0 $umol_{quanta}\, m^{-2}\, s^{-1}$, stomatal ratio = 0.5, flow = 500 $\mu mol\, mol^{-1}$, and a reference CO_2 chamber concentration = 400 $\mu mol_{CO2}\, mol^{-1}$. Measurements were logged when stability had been reached, deemed stable based on real time graphics in which the slope of the line was near zero for each variable measured on the y-axis and time on the x-axis. When recording an observation, an average was calculated for respiration, conductance and transpiration that included 15 s of gas-exchange rates. This average was the value used in subsequent analyses.

One newly opened flower per treatment group was measured each day during the duration of the experiment. This resulted in gas exchange measurements being recorded for 219 flowers.

2.7. Fitness

2.7.1. Components

We estimated fitness by collecting mature fruits. Fruits were categorized as aborted or mature based on the swollen form of the ovary (Fig. 1). In total, 192 mature fruits and 31 aborted fruits were collected at the end of the growing season. A logistic regression was used to analyze the effect of treatments on the likelihood of obtaining a mature fruit (pooling all groups and genets). Those fruits deemed mature were further dissected. Seeds within fruits were categorized as either aborted

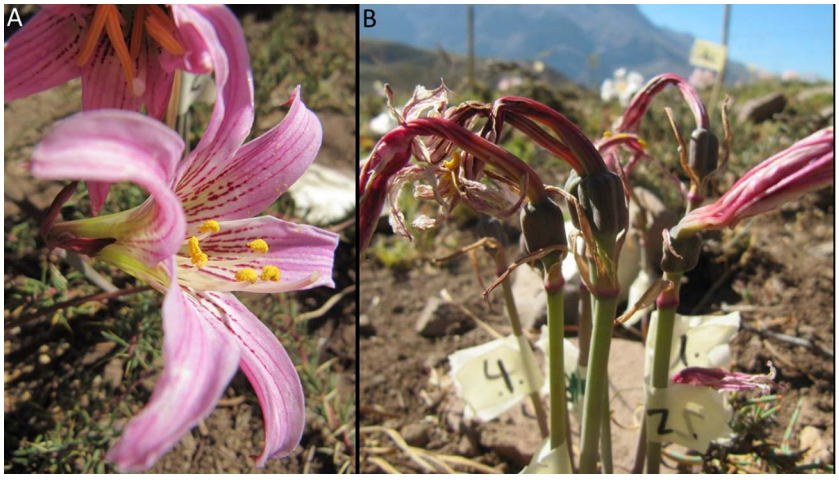

Fig. 1. Open flower (A) and swollen ovaries, showing maturing fruits (B; 7 pedicels within one genet displayed) of *Rhodolirium montanum*.

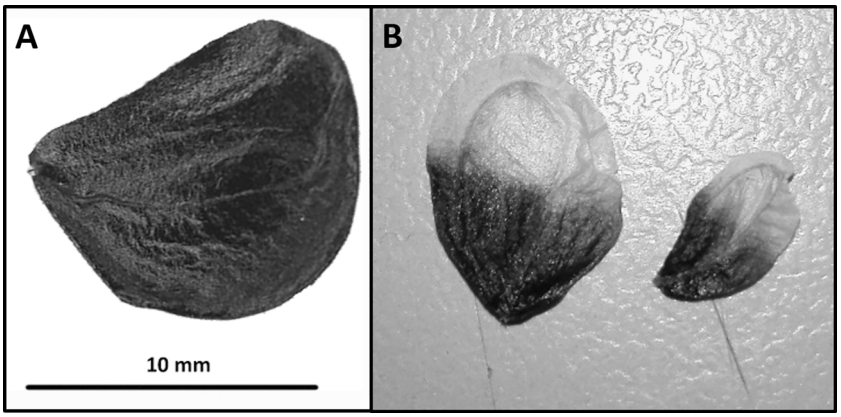

Fig. 2. Fully developed mature seed with its large black wing (A) and the two poorly developed smaller seeds (B) deemed aborted.

or mature based on morphological ripeness (Fig. 2). Two fitness components were calculated, total seed production included both aborted and mature seeds and seed set calculated as the number of mature seeds out of total seeds produced. Data for these two components were analyzed using mANCOVAs. Seed set was arcsine square-root transformed prior to analysis to meet assumptions of normality. Fixed effects included Water, Temperature and Water x Temperature. Treatment group and Genet (group) were included as random effects. In addition to the main treatment effects, flower duration was included as a covariate and permitted to interact with the fixed effects.

2.7.2. Flower duration and fitness

A subset of plants for which gas-exchange data were collected were further assessed, looking for links between flower physiology and flower duration. Separate mANCOVAs were performed in which one gas-exchange trait was treated as a covariate and the number of days a flower was open, total seed production and seed set were the dependent effects. Seed set was arcsine square-root transformed prior to analysis. Significant effects with the covariate were followed up with simple linear regression.

3. Results

3.1. Ambient environment

3.1.1. Experimental conditions

Our field manipulations created four gradated environments: −water +OTC, −water −OTC, +water +OTC, +water − OTC. In general, plants in the open-top chambers experienced hotter, drier environments as shown by a significant Temperature effect for average and maximum temperature and minimum relative humidity (Table 1 and Table Supplementary 1).

3.1.2. Soil volumetric water content

Soil moisture was significantly affected by the day of Year ($P > 0.0001$, Supplemental Table 2, Supplemental Fig. 1). On 24 January, there was an unseasonal rain event that increased soil water content that then remained at levels higher than at the beginning of the experiment. This likely ameliorated the Water effect somewhat. There was a trend, however, for soil to have higher volumetric water content in +water treatments compared to –water treatments (LSMeans ± 1SE, 0.96 ± 0.03 vs 0.89 ± 0.03%, respectively; $P = 0.1$; Fig. 3).

3.2. Flower duration

Flower duration was similar across treatments with a tendency for flowers to endure longer under the supplementally watered treatments (LSMean ± 1SE; +water 4.5 ± 0.1 days vs −water 4.2 ± 0.1 days; $P = 0.068$; Fig. 4, Supplementary Table 3). If flower duration, however, is broken into its components − flower opening and flower closing – then a significant Temperature × Water effect and a main effect of Temperature is apparent. Under supplementally watered conditions, augmenting temperature had a much smaller effect than under the –water treatment, flowers opened and closed several days earlier under warmed compared to ambient conditions (Fig. 4), which was also apparent across water treatments (LSMean ± 1SE, Open DoY +OTC 25.4 ± 0.9 vs −OTC 30.4 ± 0.7 days; Close DoY +OTC 29.6 ± 0.8 vs −OTC 34.6 ± 0.7 days).

3.3. Fitness components

3.3.1. Fruit production

Augmenting temperature negatively impacted fruit production. Of the original 486 flowers marked, we were able to collect fruit from 267

Table 1
Average ± 1SD* ambient environmental conditions during the experiment at 15 cm above surface level under two experimental treatments: watering and temperature. Different letters indicate significant pair-wise differences based on a two-way full-factorial ANOVA; for an interaction effect, the ANOVA was followed by a Tukey's Honest Significant Difference test. No letters indicates that the fixed effect term was not significant; there was no difference between the levels. Minimum relative humidity values were $\log_{10}$ transformed prior to analysis.

Water	Temp.	Temperature (°C)			Relative humidity (%)		
		Min	Mean	Max	Min	Mean	Max
−water	−OTC	6 ± 1	15 ± 3	28 ± 4^{c}	27 ± 12	58 ± 12	88 ± 8
	+OTC	5 ± 2	18 ± 3	37 ± 6^{a}	19 ± 12	54 ± 13	87 ± 9
	x	5 ± 2	16 ± 3	32 ± 7	23 ± 13	56 ± 13	88 ± 9
+water	−OTC	6 ± 2	16 ± 3	32 ± 5^{b}	24 ± 13	56 ± 13	87 ± 9
	+OTC	5 ± 2	18 ± 3	35 ± 6^{a}	22 ± 15	55 ± 14	86 ± 9
	x	6 ± 2	17 ± 3	34 ± 6	23 ± 14	56 ± 13	87 ± 9
x	−OTC	6 ± 2^{a}	16 ± 3	30 ± 5^{a}	26 ± 12^{a}	57 ± 12	87 ± 8
x	+OTC	5 ± 2^{b}	18 ± 3	36 ± 6^{b}	20 ± 14^{b}	55 ± 14	87 ± 9

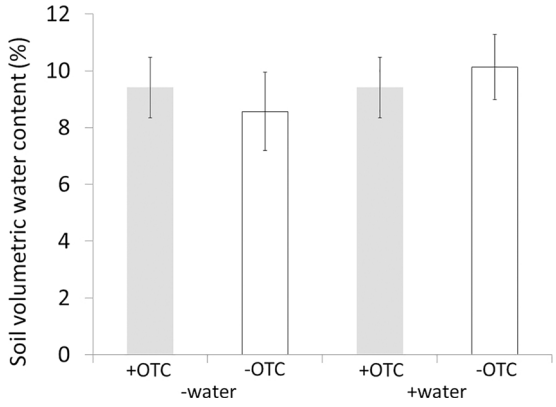

Fig. 3. Mean ± 1 SE of volumetric water content measured near focal plants under ambient (−) or augmented treatments (+) for water and temperature.

Fig. 4. Flower duration from opening to closing under treatment conditions: −water +OTC (solid grey line, grey filled marker), −water −OTC (solid black line, black filled marker) +water +OTC (dashed grey line, open marker) and +water −OTC (black dashed line, open marker).

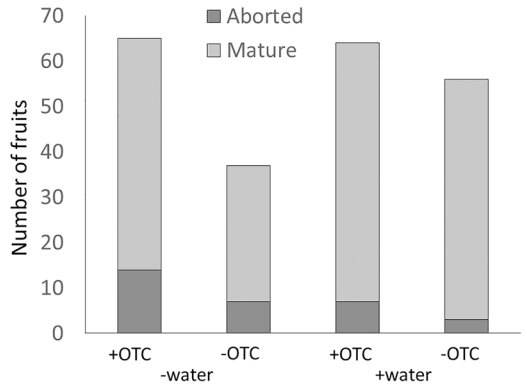

Fig. 5. Mature and aborted fruits produced by flowers hand-pollinated and grown under Water and Temperature treatment conditions.

of these. Fruits were more likely to abort for +OTC plants compared to −OTC (Temperature P = 0.01; Supplementary Table 4; Fig. 5); only 79% of flowers maturing within the OTCs reached maturity compared to 92% of the flowers outside a chamber.

3.3.2. Seed production

Plants that were subjected to experimental warming were more likely to have a reduction in fitness components that pertained to seeds. Under ambient conditions, the total number of seeds produced increased with flower duration; however, under warmed conditions, seed production decreased with flower duration (Flower duration x Temperature, P = 0.0044; Supplementary Table 5, Supplementary Fig. 2). Seed set declined as flowers remained open under −water +OTC but increased with flower duration under other conditions (Flower duration × Temperature × Water P-value = 0.0151; Supplementary Table 5; Supplementary Fig. 3).

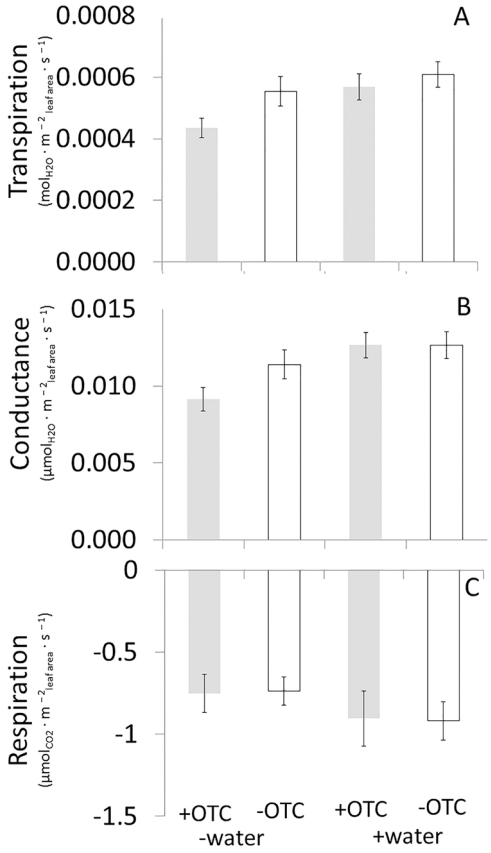

Fig. 6. Tepal gas exchange (panels A. Transpiration, B. Conductance and C. Respiration) under two treatments: Water and Temperature.

3.4. Gas exchange

3.4.1. Experimental plants

Conductance and transpiration rates were higher in plants that were supplementally watered during the experiment compared to those with no supplemental water, independent of temperature treatment (Supplemental Table 6; Fig. 6).

Respiration, however, was not significantly affected by treatments (all P > 0.3). In addition, a simple linear regression in which groups were pooled, showed very little effect of respiration on total seed production (r-square = 0.03; P > 0.1) or seed set (r-square = 0.007; P > 0.5; analyses not shown).

3.4.2. Flower duration

Since transpiration and conductance are highly correlated with one another (r = 0.88, P < 0.0001) and conductance was more strongly affected by Water compared to Temperature (F-value 7.4 vs 2.0, respectively; Supplemental Table 6), only conductance was used in models focused on gaining insights into flower duration and fitness; additionally because treatments did not significantly affect respiration (all P > 0.3), it was not used in subsequent models.

There was no indication that conductance affected flower duration either as a covariate in a mANCOVA (P > 0.9; Supplemental Table 9) or as the independent effect in a simple linear regression (r-square = 0.004; analysis not shown).

3.4.3. Fitness components

In general, raising temperature had a negative impact on fitness, but

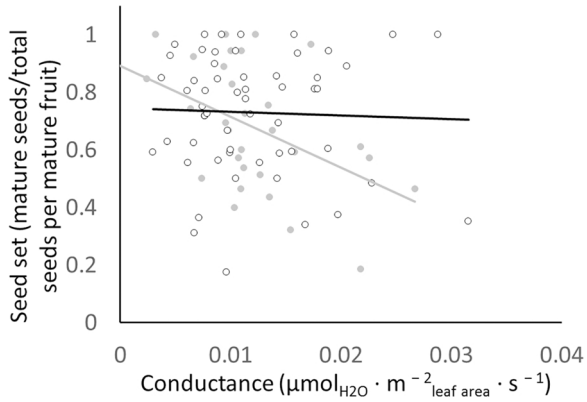

Fig. 7. Bivariate plot of tepal conductance and plant seed set (untransformed displayed but analysis on transformed data), comparing temperature treatments, +OTC (grey filled markers, solid grey line) and −OTC (open markers, solid black line).

the nuances of this depended on the water treatment, the particular estimate and tepal physiology. Total seed production for this subset of plants that also had gas exchange measured was significantly affected by Temperature ($P = 0.01$) but no other (Supplementary Table 8). Seed set, however, was significantly affected by Conductance and the interaction effect Temperature × Conductance (both $P < 0.03$). As conductance rates increased, seed set dropped significantly for plants inside OTCs; no discernible pattern, however, was detected for plants under ambient conditions (Fig. 7).

4. Discussion

Variation in floral traits is thought to be the result of optimization for pollination success, florivore escape and maintenance costs (Ashman and Schoen, 1996; Galen, 1999) however, studies on the physiology of flowers are still very rare, with most studies focused on economically or agriculturally relevant species (e.g. Azad et al., 2007; Blanke and Lovatt, 1993; Liu et al., 2017; Trolinder et al., 1993), there being few on wild species (Chapotin et al., 2003; Lambrecht, 2013; Lambrecht et al., 2011). Consequently, our knowledge of the physiological underpinning of important adaptive traits such as flower longevity and the consequences of maintaining flowers open for long periods of time on fitness is poor.

The change we induced with the OTCs was similar to that for other chamber experiments for this population. Mean temperature difference between −OTC and +OTC in the −water treatment, was ~3 °C, similar to the 3.1 °C increase reported by Pacheco et al. (2016). The water addition was a unique treatment, and did not appear to significantly affect relative humidity or temperature (Table 1), perhaps, in part, being ameliorated by the 24 January rain event. An additional treatment that prevents ambient precipitation would have contributed to our insight on how plants respond to low water availability in this system.

Overall, our results suggest that water is likely to be a more limiting factor than temperature on flower duration in *R. montanum*. While Pacheco et al. (2016) showed an average difference in flower longevity between warmed and ambient flowers of 1.2 ± 0.2 days, we did not find strong evidence of a Temperature effect. This disparity could be due to the timing of placement of the OTCs; in the earlier study, chambers were placed over experimental plants well before buds formed and while plants still had their leaves (Pacheco et al., 2016). To better understand how *R. montanum* is likely to respond to future warming and drying, we suggest permanent treatments over the entire year. The Pacheco et al. (2016) study failed to explain site differences in flower longevity, considering measurements for the entire flowering season, as a product of temperature, which is in agreement with our results. Our data suggest that water limitation and or its interaction with temperature are more important than temperature per se in determining flower longevity in the large flowers of *R. montanum* and are in accordance with the significant interannual site variation in potential flower longevity found by Pacheco et al. (2016). Our results are significant, as previous work on flower longevity in alpine environments has focussed heavily on the effect of temperature. Indeed. altitudinal increases in flower longevity on mediterranean-type climate mountains, where precipitation decreases strongly with decreasing elevation, could at least in part be due to the effect of differences in water availability.

While temperature had no significant effect on flower duration, higher temperature advanced flowering by several days. This phenomenon has been seen in other high altitude plants and may be a common reaction to warming global temperatures (Körner and Basler, 2010; Parmesan et al., 2003). For 60 species observed over 39 years, CaraDonna et al. (2014) found first flowering on average advanced by 3.3 ± 0.24 d per decade. Quense (2011) (see also Falvey and Garreaud, 2009) showed that temperature has already increased 1 °C over the past 30 years in the central Chilean Andes, indicating that global warming is already underway in the region. For *R. montanum*, the advancement of flowering may well lead to a mismatch between pollinator presence and stigma receptivity as predicted for other species (Memmott et al., 2007). Upper populations of *R. montanum* already experience pollen limitation (Arroyo et al., 2017). Thus, a mismatch between pollinator availability and stigma receptivity is likely to exacerbate this, especially in upper populations, as temperatures continue to rise. Interestingly of the 486 flowers marked, 35 did not open and of these 23 were in the most stressful treatment (−water +OTC). This, suggests that a lack of flower opening in addition to pollination mismatches, could exacerbate the negative effects of climate change on fitness in the central Chilean Andes where drier conditions are expected (Garreaud, 2011).

Under ambient conditions, the total number of seeds produced increased with flower duration; however, under warmed conditions, seed production decreased with flower duration. Seed set also declined as flowers remained open longer under the most stressful treatment (-water +OTC). The ambient condition results cannot be explained by a pollination effect, as all flowers were hand-pollinated. They are likely to reflect intra-population variation in the physiological conditions of individual plants affecting both flower longevity and seed set. Previous studies have revealed significant levels of abortion in *R. montanum* (Arroyo et al., 2017). Overall, these result point to an additional drain on resources under higher temperatures coming at a cost to seed fill and suggest that an increase in 3.2 °C could have importance consequences for fitness.

Physiologically, we showed that *R. montanum* flowers can respond plastically to environmental change and do so by maintaining high conductance rates when water availability is relatively high and reducing water loss when water availability declines; conductance and transpiration rates were higher in plants that were supplementally watered during the experiment compared to those with no supplemental water. However, respiration rates, did not vary with our treatment conditions. The last result is surprising as some work has shown flower respiration rates to increase with temperature (Seymour et al., 2009). Also, cool temperatures associated with lower respiration rates can prolong virgin flower lifespans in cut flowers (Çelikel and Reid, 2002; Cevallos and Reid, 2000). Our results could reflect the draw by the flower on stored resources from below-ground organs (Lubbers and Lechowicz, 1989), possibly reflecting immediate versus longer term storage feedback. A multi-year approach may help to answer this question without destroying the integrity of the root-soil connections by digging up bulbs before and after a treatment, maintaining treatments across years and assessing reproduction for carry-over effects. The flowering stem is the only other above ground organ at the time of flowering and is green. It is unknown how much it might contribute to the plant's gas exchange. There was no indication that conductance affected flower duration, suggesting that this aspect of flower

physiology and longevity may be independent of one another in our species.

As conductance rates increased, seed set dropped significantly for experimentally warmed plants; no discernible pattern, however, was detected for plants under ambient conditions (Fig. 7). This indicates two things. One, evaporative cooling is not likely to be an adaptive feature for this population because there was not a positive correlation between conductance and seed set. Two, maintenance costs of flowers under higher temperatures could constrain longer flower duration by negatively impacting fitness.

Plants without water addition had flowers that tended to close sooner with lower rates of conductance. As conductance decreased, seed set tended to increase in these plants. This pattern indicates that plants appear to be adapted for optimal fitness, responding plastically under stressful conditions, warm and dry.

5. Conclusions

We asked if plants respond to the ambient environment by reducing flower maintenance costs through reduced gas exchange (particularly conductance, transpiration and respiration) and shortening flower longevity under more stressful conditions, artifically warmed without supplemental watering. Our study showed that flower longevity tends to decrease under drier conditions, but the effects on bud opening and flower closing were even stronger, with the first reduced and the second advancing under higher temperatures without supplemental watering. Gas exchange also decreased under drier, warmer conditions, limiting water loss.

We followed this by searching for evidence that plant responses might be considered adaptive, which would be indicated by those plants that minimized maintenance under more stressful conditions having higher fitness components. Those plants that did reduce flower longevity under warmed conditions had higher total seed production and seed set, supporting our prediction. These results suggest that *R. montanum* optimizes fitness by reducing flower maintenance costs and are in accordance with the resource-cost hypothesis.

The experimental conditions created in the present study are similar to the combination of an increase in temperature and reduction in precipitation predicted by climate change models for central Chile. Our data suggest that species that have relatively long-lived flowers may have reduced fitness due to maintenance costs such as water loss through gas exchange. Alpine plants may be particularly vulnerable to high temperatures and reduced water availability due to their relatively large flowers. Further experimental work looking at the combined impacts of water and temperature on other species is critical as these two environmental variables are vital to plant species persistence and are changing rapidly as the effects of climate change become stronger. In addition, studies would benefit by considering the effect of environmental variables on bud opening and flower dehiscense in addition to flower longevity, as longevity per se may not be the most ecologically relevant floral trait insofar as fitness is concerned.

Contributions

LS Dudley takes primary responsibility for the integrity of the work as a whole, from inception to finished article. MTK Arroyo was 1) instrumental in the conception of the study, 2) took some data but was mainly instrumental in providing support in data collection, and 3) aided in the interpretation, drafting of the article with an important contribution to the intellectual content of the article in the context of her current Fondecyt grant. MP Fernandez-Murillo 1) helped to make changes in the field to improve the design of the study, 2) was largely responsible for data acquistion, and 3) aided in interpretation of data and drafting of the article.

Funding

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico [Fondecyt Grant 1140541] (URL: www.conicyt.cl/fondecyt/) to MTKA, Iniciativa Cientifica Milenio [Grant ICM-MINECON P05-002] (URL: www.iniciativamilenio.cl/) to the Instituto de Ecología y Biodiversidad and Programa de Financiamiento Basal [Grant PBF-23] (URL: www.conicyt.cl/) to the Instituto de Ecología y Biodiversidad. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

Permission to work on the three sites was granted by Ricardo Margulis, General Manager of the private Valle Nevado Ski resort and Humberto Gallardo, who manages private land below the ski complex. Valentina Riffo and several other field assistants provided hours of data collection in difficult terrain and conditions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2018.02.015.

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